National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

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RIVM letter report 2021-0217
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Synopsis

A literature study on the toxicokinetics of structural analogues of the mycotoxin deoxynivalenol

When eating grain and cereal products like bread and biscuits, people can ingest substances made by moulds. We call these substances mycotoxins. If the concentration of mycotoxins is too high, this can be harmful to our health and cause problems such as diarrhoea and vomiting.

RIVM previously created a model that can calculate how much of one type of mycotoxin (deoxynivalenol) we ingest via food and then excrete through urination. This model is unique, because it only requires measurements in the urine. Mycotoxins that cannot always be measured in grain and cereal products, can be measured in urine. The amounts of mycotoxins that we ingest are currently estimated using information about the level of mycotoxins in grain and cereal products in combination with the amounts that we eat.

RIVM would like to further develop the model for similar mycotoxins in food. The institute therefore conducted a scientific literature review to find out which mycotoxins the model could also be used for. There are two: T-2 toxin and HT-2 toxin. RIVM can calculate the relationship between the amount we ingest and excrete for these mycotoxins as well.

This study involved gathering information about how different mycotoxins 'behave' in animals, such as how quickly they are broken down in the body and end up in the urine. There is no such knowledge about these mycotoxins in humans.

This study was carried out on behalf of the Netherlands Food and Consumer Product Safety Authority (NVWA). The NVWA monitors the concentration of mycotoxins in cereals and other food products through random sampling. The model can provide additional insight into the concentrations that people are exposed to.

Keywords: mycotoxins; kinetics; human biomonitoring

Publiekssamenvatting

Literatuuronderzoek over de toxicokinetiek van structuuranalogen van de mycotoxine deoxynivalenol

Mensen kunnen via graanproducten, zoals brood en koekjes, stoffen binnenkrijgen die gemaakt zijn door schimmels. Deze stoffen noemen we mycotoxinen. Als de concentratie mycotoxinen te hoog is, kan dat schadelijk zijn voor de gezondheid. Dit kan bijvoorbeeld diarree of overgeven veroorzaken.

Het RIVM heeft eerder een model gemaakt dat kan berekenen hoeveel van één soort mycotoxine (deoxynivalenol) we via voedsel binnenkrijgen en vervolgens uitplassen. Dit model is bijzonder omdat er alleen metingen in urine voor nodig zijn. In urine kun je mycotoxinen aantonen die in graanproducten niet altijd meetbaar zijn. Op dit moment worden de hoeveelheden mycotoxinen die mensen binnenkrijgen geschat met informatie over gemeten gehaltes in graanproducten, in combinatie met hoeveel ervan wordt gegeten.

Het RIVM wil het model nog verder ontwikkelen om het bruikbaar te maken voor andere mycotoxinen in voedsel. Het RIVM heeft daarom in de wetenschappelijke literatuur gezocht voor welke mycotoxinen dat mogelijk is. Dat blijkt voor twee soortgelijke mycotoxinen te kunnen: T2 toxine en HT2 toxine. Ook voor deze mycotoxinen kan het RIVM de relatie berekenen tussen de hoeveelheid die we ervan binnenkrijgen op basis van wat er via urine wordt uitgescheiden.

Voor dit onderzoek is informatie verzameld hoe verschillende mycotoxinen zich in dieren ‘gedragen’. Bijvoorbeeld hoe snel zij in het lichaam worden afgebroken en in de urine terechtkomen. Deze kennis bestaat niet over het gedrag van deze mycotoxinen in mensen.

Dit onderzoek is gedaan in opdracht van de Nederlandse Voedsel- en Warenautoriteit (NVWA). De NVWA controleert met steekproeven de concentratie mycotoxinen in granen en andere voedselproducten. Het model kan meer inzicht geven in de concentratie waar mensen aan blootstaan.

Kernwoorden: mycotoxinen; kinetiek; humane biomonitoring

Contents

Summary

The relation between the dietary intake and urinary excretion of the mycotoxin deoxynivalenol (DON) and its metabolite deoxynivalenol-15-glucuronide in humans has recently been modelled (van den Brand et al., 2021). It is common knowledge that there are mycotoxins that are structurally similar to DON. The aim of this report was to investigate for which structural analogues of DON the relation between dietary intake and urinary excretion can also be modelled, to estimate the dietary exposure to these mycotoxins in humans in future biomonitoring studies.

DON is a type B trichothecene and a literature study was performed focusing on other type B trichothecenes, namely nivalenol (NIV) and fusarenone-X (FusX; 4-acetyl-nivalenol) and type A trichothecenes T2 and HT2 toxin. Specific information related to the absorption, distribution, metabolism and excretion of these mycotoxins in humans and animals was identified.

NIV is mainly excreted via feces and only a small fraction of the ingested NIV is recovered (as its metabolite) in urine. This appears in contrast to FusX, where more of the ingested compound is recovered in urine than in feces. Moreover, FusX is partly excreted by the kidney as NIV. T2 toxin can be metabolized to HT2 toxin, and both compounds appear to be excreted via urine mainly as conjugated metabolites. No data on the metabolism of T2 and HT2 toxin in humans was identified.

NIV in urine is not only an elimination product of ingested NIV, but also of the ingested FusX. Therefore, excreted NIV cannot solely be attributed to the dietary intake of NIV. Nor can excreted NIV be attributed to the dietary intake of the sum of NIV and FusX because there is a presumed, but unknown, difference in human kinetics of NIV and FusX. The latter is also true for excreted FusX. Therefore it is uncertain to what extent the excreted total of NIV and FusX could serve as a measure for the total dietary intake of NIV and FusX. In addition, there is not one health-based guidance value (HBGV) for this group of toxins.

For T2 and HT2 toxin the situation is different. There is a HBGV for the group of T2 and HT2 toxin (including their modified forms). Although no human urinary clearance rates are known, based on data from animal studies it appears that total urinary recovery of T2 and HT2 toxin, and their metabolites, could reflect the total of ingested T2 and HT2 toxin.

Therefore, T2 and HT2 toxin were selected to model the relation between the intake and excretion of T2 and HT2 toxin (including metabolites) in humans. A toxicokinetic model for T2 and HT2 toxin could be used to estimate the total intake of these mycotoxins and will support future risk assessment based on human biomonitoring studies.

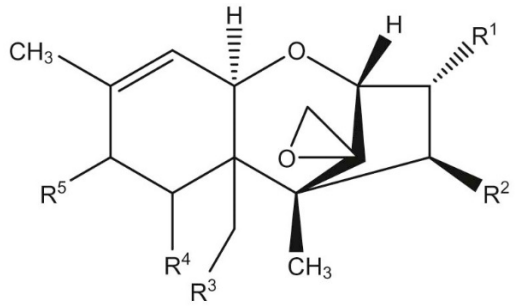
1 Introduction

Human biomonitoring (HBM) can be used to assess the exposure to mycotoxins by analyzing human matrices, such as urine or blood, for mycotoxins and their metabolites. These data can be used to directly assess the risk of the exposure to compounds, if HBM guidance values are available. These data can also be used to estimate the external (dietary) exposure to compounds and assess the risk based on external health-based guidance values (HBGV). In order to also quantitatively estimate the external exposure, information on the toxicokinetics of the respective mycotoxin is required. Only when the relevant excretion products, the fraction of the parent compound that is excreted and the excretion route(s) are known, HBM may be used as a tool to assess the external exposure. However, for many mycotoxins kinetic models that reflect this information are not available.

An advantage of HBM is that it also takes into account the exposure to the so called 'masked' or 'hidden' forms of mycotoxins. These are (plant) metabolites or bound forms of the parent mycotoxins, which are often not included in routine food monitoring programs (Slobodchikova et al., 2019). But, these bound forms of mycotoxins can be converted back to their parent mycotoxins in the human gut, thereby adding to the exposure of the parent compound (Gratz et al., 2017; Mengelers et al., 2019). The exposure to these masked mycotoxins can even attribute up to 30% of the parent compound (EFSA, 2014), but is often not included in 'traditional' dietary exposure assessments using data on the occurrence in foods and food consumption data.

The relationship between the total intake of the trichothecene deoxynivalenol (DON) and the excretion of its main metabolite deoxynivalenol-15-glucuronide (DON15GlcA) was assessed in project 9.4.58 as commissioned by the Netherlands Food and Consumer Product Safety Authority (NVWA) (van den Brand et al., 2021). This was done by analyzing all urine samples over 24 hours from 49 participants in the Norwegian EuroMix HBM study (Husøy et al., 2019). Of these participants, the dietary exposure was also estimated in the traditional way by using the available food records. By doing so, the relation between the intake of DON and the excretion of its main metabolite DON15GlcA in urine could be investigated.
The aim of this literature search is to identify relevant information on the metabolism and toxicokinetics of structural analogues of DON, the trichothecenes nivalenol (NIV), fusarenone-X (FusX) and T2 and HT2 toxin (table 1). This information was used to assess whether the relation between intake and excretion can be modelled for these mycotoxins using HBM.

Table 1 Structural analogy of the trichothecenes, from Broekaert et al. (2015). DON: deoxynivalenol; DON3G; deoxynivalenol-3-glucoside; 15ADON: 15-acetyl-deoxynivalenol; 3ADON: 3-acetyl-deoxynivalenol; DON3GlcA: deoxynivalenol-3-glucoronide; DON15GlcA: deoxynivalenol-15-glucuronide; NIV: nivalenol; FUS-X: fusarenone-X; T-2: T2 toxin; HT-2: HT-2 toxin; T-2G: T2 toxin-glucoside; HT-2G: HT2 toxin-glucoside; OAc: acetoxy group.



Mycotoxin	***R¹***	***R²***	***R³***	***R⁴***	***R⁵***
DON	OH	H	OH	OH	O
DON3G	$C_6H_{11}O_6$	H	OH	OH	O
15ADON	OH	H	OAc	OH	O
3ADON	OAc	H	OH	OH	O
DON3GlcA	$C_6H_9O_7$	H	OH	OH	O
DON15GlcA	OH	H	$C_6H_9O_7$	OH	O
NIV	OH	OH	OH	OH	O
FusX	OH	OAc	OH	OH	O
T2	OH	OAc	OAc	H	$C_5H_9O_2$
HT2	OH	OH	OAc	H	$C_5H_9O_2$
T2G	$C_6H_{11}O_6$	OAc	OAc	H	$C_5H_9O_2$
HT2G	$C_6H_{11}O_6$	OH	OAc	H	$C_5H_9O_2$

Here, we focused on identifying information regarding *absorption and distribution*, the uptake of the mycotoxins from the gastro-intestinal tract into the blood and further distribution of the mycotoxins into the body; *metabolism*, the transformation and degradation of the mycotoxins in order to detoxify and excrete the parent compounds and, *excretion,* the elimination of the mycotoxins (and their metabolites) in urine or feces (Cavret & Lecoeur, 2006).

Only few studies have been published on the absorption, distribution, metabolism and excretion of these mycotoxins in humans. Therefore, relevant *in vivo* studies with laboratory animals or farm animals were also considered. Studies with pigs are especially relevant here, since the gastro-intestinal tract, the renal system and the cardiovascular system of these animals closely resemble those of humans (Broekaert et al., 2015; Henze et al., 2019; Schelstraete et al., 2020; Svendsen, 2006).

2 Methods

The European Food Safety Authority's (EFSA) most recent scientific opinions of NIV and T2 and HT2 toxin were considered a starting point to obtain information on the metabolism and toxicokinetics of the mycotoxins (EFSA, 2017a, 2017b). No EFSA scientific opinion has been dedicated to FusX. An additional literature search was conducted to identify studies with new information that were not considered in the EFSA opinions. This additional search was designed with a focus on the toxicokinetics of the relevant mycotoxins. Studies published one year before the release of the respective EFSA opinions were considered, except for FusX, for which no publication time limit was applied. The online database Embase was used to search the literature and the Embase search strings were developed in cooperation with an RIVM information specialist. See Annex A for the search strings for FusX, NIV and T2 and HT2 toxin.

The identified literature was exported to an Endnote database in which the duplicate studies were removed and the PDFs of the studies were retrieved. All titles and abstracts were screened to select relevant studies. Studies that were deemed not relevant, and which were thus excluded from this literature screening were: conference abstracts only, non-English articles, studies related to plant health or bacteria, studies not related to these specific mycotoxins, studies related to the natural contamination with unknown levels of mycotoxins or inoculation with *Fusarium* spp., studies related to the occurrence of the mycotoxins, studies related to mycotoxin decontamination strategies, *in vitro* studies but not *in vitro* metabolism studies with microsomal fractions, and *in vivo* studies not related to the elimination of the mycotoxins or *in vivo* studies not considering laboratory or farm animals. Full texts were subsequently screened to identify relevant information related to the metabolism of the mycotoxins.

3 Results

3.1 Nivalenol

Nivalenol (NIV) is, like deoxynivalenol (DON), a mycotoxin from the trichothecene type B family. NIV is predominantly found in wheat, oat, barley and maize (EFSA, 2013), in approximately 14% of all grains in Europe (Schothorst & van Egmond, 2004). NIV can subsequently be processed into food products such as bread and beer (EFSA, 2013; Kongkapan, Polapothep, et al., 2016).

Exposure to high levels of NIV can be a concern for human health. EFSA derived an health-based guidance value (HBGV) of 1.2 microgram per kilogram body weight per day for the intake of NIV (EFSA, 2013, 2017a). This is based on the most critical effect of NIV in laboratory animal studies; immune and hematological effects i.e. reduced white blood cell count (Takahashi et al., 2008). In contrast to deoxynivalenol, no maximum limits for nivalenol in agricultural products and foodstuffs have been set in the EU Commission Regulation (EC) No 1881/2006 Setting maximum levels for certain contaminants in foodstuffs.

Also, a masked form of NIV has been reported in artificially infected wheat grains. Nivalenol-glucoside (NIVGlc) is a glucosylated plant metabolite of NIV and is estimated to add between 15%-50% to the exposure of NIV from grains (EFSA, 2017a; Nakagawa et al., 2011).

3.1.1 Toxicokinetics

Absorption & distribution

NIV is, similar to DON, rapidly absorbed in the small intestine (Cavret & Lecoeur, 2006). NIV was detected in the plasma of pigs within approximately 20 minutes after oral exposure (Hedman et al., 1997). Hedman et al. (1997) report that 7.5 hours after oral administration of NIV, about 11-43% of the dose was absorbed. Plasma peak concentrations of NIV were observed after 2.5-4.5 hours after feeding in pigs (Hedman et al., 1997), 2.5 hours after feeding broiler chickens (Kongkapan, Giorgi, et al., 2016) and one hour after oral administration in mice (Poapolathep et al., 2003a). The oral bio-availability of NIV is estimated at 4% in broiler chickens (Kongkapan, Giorgi, et al., 2016). The absorption of NIV appears to be slower than that of DON, as it is a less lipophilic compound (Cavret & Lecoeur, 2006).

Metabolism

NIV is generally not extensively metabolized in the body (Cavret & Lecoeur, 2006; Kongkapan, Polapothep, et al., 2016). In urine and feces of rats, de-epoxy-nivalenol (DNIV) was identified after a high dose of 5 milligram NIV per kilogram body weight (Onji et al., 1989). This metabolite was also reported in the feces of laying hens (Garaleviciene et al., 2002). NIV appears to be metabolized to DNIV in pigs only after long-term exposure (Kongkapan, Polapothep, et al., 2016). It is believed that the de-epoxydation of NIV is a product of the gastrointestinal microbiota, rather than produced by the liver (EFSA, 2017a; Hedman et al., 1997). However, human microbiota were not able to transform NIV

into DNIV, after incubation of NIV with human feces (Sundstol Eriksen & Pettersson, 2003). Recently, the presence of NIV glucuronides were reported after incubation of NIV with human microsomes, which indicates a type II metabolism of NIV via glucuronidation (Slobodchikova et al., 2019). These metabolites have not been identified in human samples, likely as a result of the polarity of NIV and its metabolites in combination with relatively high detection limits (Slobodchikova et al., 2019).

Excretion
All trichothecenes are generally quickly and abundantly eliminated, without any apparent accumulation in the organism (Cavret & Lecoeur, 2006; Eriksen & Pettersson, 2004). Although a small accumulation of NIV has been reported in hens (Cavret & Lecoeur, 2006), this has not been demonstrated for other animal species. In laying hens, NIV and DNIV accounted for approximately 10% of the total dose in feces (Garaleviciene et al., 2002). In pigs, the excretion of NIV via feces was estimated at 67%, whereas the excretion via urine was 17% after oral administration (Cavret & Lecoeur, 2006; Hedman et al., 1997). In a rat study, 80% of the dose was recovered in feces as DNIV and 7% as NIV after oral administration. In urine, only 1% of the dose was recovered as DNIV and 1% as NIV (Onji et al., 1989). In a study with radioactive labeled NIV and FusX in mice, most NIV was detected in feces after oral administration (three times more than in urine), in contrast to FusX (five times more in urine than in feces). Absolute percentages of elimination were not given (Poapolathep et al., 2003a). The elimination half-life of NIV was estimated at 14 hours in a two-compartment model after oral administration of NIV in mice in that study. The elimination half-life in broiler chickens was estimated at 2.5 hours after oral administration of NIV (Kongkapan, Giorgi, et al., 2016).

3.1.2 Conclusion

It appears that NIV is mainly excreted via feces and only a small fraction of the administered NIV is recovered (as DNIV) in urine. This is however highly variable among the different laboratory animal species. In humans, it is expected that a small, but significant fraction of NIV can be recovered in urine, considering that this was also the case for pigs.

3.2 Fusarenone-X

Fusarenone-X (FusX, or 4-acetylnivalenol) is a mycotoxin that is a member of the trichothecene type B group, like DON and NIV. FusX is also found in grains and grain-based products, albeit to a lower extent than DON and NIV (IARC, 1993; Juan et al., 2013; Vanheule et al., 2014). FusX is often detected simultaneously with other mycotoxins in food, mainly in Europe and Asia, but also in Sub-Saharan Africa (Aupanun et al., 2017; Chilaka et al., 2017). The presence of FusX, whether or not together with DON or NIV, has also been reported in Belgium in various raw grains, food and feed (Vanheule et al., 2014). FusX was detected in three out of 20 breakfast cereal samples, with an average concentration of 796 microgram per kilogram sample. FusX was also detected in two out of 25 bread samples, with an average concentration of 505 microgram per kilogram sample.

Where health-based guidance values are derived for DON and NIV, and maximum limits in agricultural products and foodstuffs are in place for DON, these are not established for FusX Commission Regulation (EC) No 1881/2006 Setting maximum levels for certain contaminants in foodstuffs.

There are indications that FusX, like DON and NIV, can be of concern for human and animal health. Reported effects after exposure to FusX are, among others, immunosuppression and intestinal (glucose) malabsorption. The underlying mechanism of action may be a FusX-induced ribotoxic stress response (Aupanun et al., 2016). This can result in an inhibited synthesis of protein and DNA in the cells. That is possibly why FusX mainly affects organs with a relative quick cell cycle division, such as the thymus, spleen, small intestine, testis, skin and hematopoietic tissue (IARC, 1993). In addition, a metabolite of FusX is NIV (see 3.2.1), and this may play a role in the adverse effects caused after exposure to FusX. Yet, more studies are needed to reveal the mode of action of FusX toxicity.

A masked form of FusX, fusarenone-X-glucoside (FusXGlc), has also been reported in artificially contaminated wheat grain. FusXGlc is a glucosylated plant metabolite of FusX (Nakagawa et al., 2011). The authors of that study estimate that over 15% of FusX in wheat grain is accounted for by a masked form of FusX, and is consequently not found in analysis of FusX.

3.2.1 Toxicokinetics

Absorption & distribution

In the literature, it has been reported that FusX is easily absorbed from the gastro-intestinal tract in several animal species, and the reported oral bio-availability was highest in pigs (Broekaert et al., 2015). The oral bio-availability of FusX was estimated at 75% in pigs, 10% in broiler chickens and 20% in ducks (Poapolathep et al., 2008; Saengtienchai et al., 2014). The absorption rate and efficiency of FusX was higher than that of NIV in mice (Cavret & Lecoeur, 2006; Poapolathep et al., 2003a). The highest plasma concentrations of FusX were found at 30 minutes after oral administration of FusX in mice, with comparable doses (Poapolathep et al., 2003a)**.** In pigs, plasma concentrations of FusX and NIV were found already five minutes after oral administration (Saengtienchai et al., 2014).

Metabolism

In pigs, FusX was found in the liver, kidney and spleen of the animals three hours after oral administration (Saengtienchai et al., 2014). FusX is subsequently metabolized quickly via deacetylation to its metabolite NIV in pigs, mice, broiler hens and ducks (Ohta et al., 1978; Poapolathep et al., 2008; Poapolathep et al., 2003a; Saengtienchai et al., 2014; Slobodchikova et al., 2019). This metabolization occurs mainly in the liver, although also to a lesser extent in the kidneys (Poapolathep et al., 2008; Poapolathep et al., 2003b; Saengtienchai et al., 2014). It has also been reported that FusX, via its metabolite NIV, can cross the placenta and thus reach the fetus in mice (Aupanun et al., 2017). Information regarding the metabolism of FusX in humans has however not been reported. It does appear from an *in vitro* study with

human liver microsomes that 54% of FusX is transformed to NIV, without the aid of phase I enzymes. FusX was hardly glucuronidated (<1%) by phase II enzymes *in vitro* (Slobodchikova et al., 2019).

Excretion
Both FusX and NIV were detected in the urine of mice, one hour after oral administration to FusX. No FusX was detected in urine of the animals after 24 hours, but NIV was still detected at 24 hours after exposure to FusX (Poapolathep et al., 2003b). The elimination half-life of FusX was in that study estimated at 38 hours using a two-compartment model after oral administration in mice. It is however not know if this biphasic excretion that was observed in mice, is also relevant to the human situation. FusX and mainly NIV were also observed in the urine and feces of pigs up to 48 hours after oral administration of FusX (Saengtienchai et al., 2014). The highest concentrations of FusX and NIV in urine were found after four and eight hours, respectively and in feces after eight hours for both matrices. It has been suggested that FusX is fully excreted, or metabolized to NIV, within 24 hours (Aupanun et al., 2017; Kongkapan, Polapothep, et al., 2016; Wu et al., 2010).

As mentioned above, in a study with radioactive labeled NIV and FusX in mice, most FusX was detected in urine (five times more than in feces) after oral administration, in contrast to NIV (three times less than in feces) (Poapolathep et al., 2003a).

3.2.2 Conclusion

FusX is partly excreted as NIV. Urinary NIV concentrations are therefore not only a product of the ingested NIV, but also of FusX. Since the urinary elimination fractions of FusX and NIV do not appear to be similar, recovered concentrations in urine can hardly be used to assess the simultaneously ingested NIV and/or FusX.

3.3 T2 toxin and HT2 toxin

T2 toxin and its major (plant) metabolite HT2 toxin are mycotoxins classified as trichothecenes type A. T2 and HT2 toxins are generally found in cereal grains such as oats, barley, maize and wheat (EFSA, 2017c; Kis et al., 2021)**.** Consequently, products in the category 'cereal flakes' and 'fine bakery wares' contributed most to exposure of T2 and HT2, in addition to other grain-based foods (EFSA, 2017c).

Upon ingestion, T2 and HT2 toxin can induce adverse effects to several organ systems such as the digestive system, nervous system, immune system and reproductive system in both humans and animals (EFSA, 2017b; Wu et al., 2020). The most sensitive adverse effect of T2 toxin was characterized as a reduction in white blood cell count (EFSA, 2017b). As T2 toxin is rapidly metabolized into HT2 toxin, and the acute toxicity of both toxins is in the same range, (part of) the toxicity of T2 toxin may be attributed to HT2 toxin (Schuhmacher-Wolz et al., 2010). Therefore, the health based guidance value for T2 toxin is grouped and includes HT2 toxin and their modified forms (EFSA, 2017b). In the EU, there are no maximum limits set for T2 and HT2 toxin in food or feed,

although the monitoring of these mycotoxins in cereals and cereal products is encouraged (Commission Recommendation 2013/165/EU). Masked forms of T2 and HT2 have also been reported as for example 3-O-glucosides of T2 and HT2 in oats and wheat (Crews & MacDonald, 2016; Lattanzio et al., 2012; Veprikova et al., 2012). In the digestive tract, these glucosides can be (partially) converted back to the parent compound by intestinal microflora, thereby adding to the exposure of T2 or HT2 toxins (Daud et al., 2020; Kasimir et al., 2020; Wu et al., 2020). It was estimated by EFSA from the study by Lattanzio et al. (2012) that an additional 10% of T2/ HT2 exposure was a result from masked forms of T2 and HT2 (EFSA, 2014).

3.3.1 Toxicokinetics

Absorption & distribution

T2 toxin is rapidly cleared in rodents, with plasma levels in mice reportedly attaining peak levels after 30 minutes and a plasma half-life of less than 20 minutes (Adhikari et al., 2017; Doi et al., 2006; SCF, 2001; Schuhmacher-Wolz et al., 2010). No information on the bio-availability of T2 toxin was identified. After absorption, it is readily distributed to the liver, the kidney and other organs without apparent accumulation (EFSA, 2017b; Matsumoto et al., 1978). Pace (1986) reported that the total recovery of radiolabeled T2 in a rat perfusion experiment was approximately 97%, with most of it found in the bile (Pace, 1986). The plasma half-life of T2 and metabolites, measured as total radioactivity, was estimated at approximately 1.5 hours in pigs, after intravascular administration of T2 (Corley et al., 1986). In broiler chickens, almost no T2 toxin was present in the plasma approximately 20 hours after oral administration, however, the absolute oral bio-availability of T2 toxin was estimated to be only 2%, whereas the bio-availability of T2-glucoside was 5 times higher (Broekaert et al., 2017). In contrast, an absolute bio-availability of 17% was reported for T2 toxin in another study with broiler chickens after multiple oral administrations (Sun et al., 2015). Also, T-2 toxin was not detectable in the analyzed tissues of orally exposed broiler chickens after 48 hours post feed-replacement with non-contaminated feed (Yang et al., 2020).

Metabolism

T2 toxin is metabolized to a number of different compounds, varying per species. One of the major phase I metabolites of T2 toxin in many species is HT2 toxin, the deacetylated form of T2 (EFSA, 2017b). Deacetylation can also occur in the gut by microbial transformation (Escriva et al., 2015). HT2 toxin can further be metabolized into 3'-OH-HT2 toxin in rat, chicken and swine liver microsomes (Yang et al., 2017). In an *in vitro* study with human cell lines, HT2 and neosolaniol were identified as major T2 metabolites and 19-hydroxy-T2, T2-triol and 4-deacetyl-neosolaniol as minor metabolites (EFSA, 2017b; Weidner et al., 2012). In *in vitro* studies using human microsomes, respectively T2-triol and HT2 toxin (Lin et al., 2017) and HT2 toxin and neosolaniol (Wu et al., 2011) were identified as major metabolites. In pigs, T2 and HT2 toxin are metabolized by cytochromeP450 (CYP) enzymes that are orthologues of the human CYP3A4, which result in 3'-OH-T2, 3'-OH-HT2 and neosolaniol (Schelstraete et al., 2020).

Most of T2 and its phase I metabolites are subsequently extensively conjugated with glucuronides by phase II metabolizing enzymes (EFSA, 2017b). HT2-3-glucuronide and HT2-4-glucuronide were identified in the urine of orally administered T2 toxin to pigs (EFSA, 2017b; Schelstraete et al., 2020). HT2-3-glucuronide appeared the major phase II metabolite in an *in vitro* study with human microsomes (Wu et al., 2011). Schelstraete et al. (2020) also summarize that the ADME characteristics of T2 are comparable between humans and pigs. After i.v. administration of radiolabeled T2 toxin in two swine, less than 30% of the total metabolites in urine were present as free compounds after 4 hours and it was estimated that around 63% of the metabolite residues in the urine were glucuronide conjugates. In addition, 20% and 40% of the total dose was recovered in urine over 4 hours after i.v. administration of T2 toxin in the two animals, with (conjugated) 3'-OH-HT2 being the major metabolite identified, followed by conjugated HT2 toxin (Corley et al., 1985).

Excretion
T2 toxin and its metabolites are excreted via both urine and feces. The excretion ratio between the two matrices depends on the species. In mice and rats, T2 toxin was excreted in feces and urine with a ratio of approximately 5:1 after oral administration. After 24 hours, 62% of the radioactivity was recovered, increasing to 69% after 72 hours. Approximately 10-12% of the total dose of radiolabeled T2 toxin was eliminated via the urine in 24 hours (Matsumoto et al., 1978). Pfeiffer et al. (1988) reported that in rats, over 95% of the orally administered radiolabeled T2 toxin was recovered after 72 hours in feces and urine, with the excretion peaking after 24 hours. Approximately 15-20% of the radioactivity was recovered in urine after 24 hours and approximately 80% in feces (Pfeiffer et al., 1988). Approximately 20% of the orally administered dose of radioactive labeled T2 was recovered in the urine of swine (Robison et al., 1979). In a lactating cow, urinary excretion of radiolabeled T2 was complete within 48 hours, accounting for about 30% of the total dose. Almost all radioactivity had been recovered after 72 hours (Yoshizawa et al., 1981).

Several studies analyzed T2 and HT2 in human spot urine samples, yet low incidences of the presence of T2 and HT2 were found (Fan et al., 2019; Gratz et al., 2020; Rodriguez-Carrasco et al., 2014). However, the glucuronidated metabolites of T2 and HT2 were not analyzed, as these standards are not commercially available. These biomarker studies from different regions did not address the dietary intake of the mycotoxins.

3.3.2 Conclusion

There is limited information available on the toxicokinetics of T2 and HT2 toxin in mammals, and even less in humans. It appears that T2 toxin is almost completely absorbed *in vivo* (in rats and mice), and its excretion is complete within 72h, reaching an almost maximum excretion after 24-48 hours, depending on the dose. Urinary T2 toxin elimination is estimated around 20% in swine after oral administration. Free T2 toxin, HT2 toxin, and HT2–4-glucuronides should be regarded as the main human T2 toxin biomarkers in the urine. Yet, no human urinary clearance rates are known.

4 Conclusion

The aim of this study was to investigate which structural analogues of DON, a type B trichothecene, can be used to model the relation between their dietary intake and their urinary excretion. Therefore, a literature study was performed on the toxicokinetics of two type B trichothecenes, namely nivalenol (NIV) and fusarenone-X (FusX), and two type A trichothecenes, namely T2 and HT2 toxin.

Unfortunately, these mycotoxins do not have unique excretion products in urine. FusX can be partly metabolised to NIV and excreted in urine as FusX and NIV (including some other metabolites). Similarly, T2 can be metabolised to HT2 and excreted in urine as T2 and HT2 (including some other metabolites).

With respect to the toxicokinetics, the urinary excretion rates of these mycotoxins are important for modelling. There is an unknown difference in the urinary excretion rates of NIV and FusX in humans. Based on animal studies it is presumed that urinary excretion rates of T2 and HT2 in humans are rather similar or dominated by the excretion rate of HT2 (and its metabolites).

With respect to the risk assessment of these four mycotoxins there is also an interesting difference between the two types of trichothecenes. Although a HBGV was derived for NIV, no HBGV has (yet) been derived for FusX. For T2 and HT2 toxin, there is a group HBGV for T2 and HT2 toxin (including their modified forms). In addition, total urinary recovery of T2 and HT2 toxin, and their metabolites, could reflect the total of ingested T2 and HT2 toxin. Consequently, the estimated total intake of T2 and HT2 can be modelled for risk assessment purposes.

Therefore, T2 and HT2 toxin were selected to model the relation between the intake and excretion of T2 and HT2 toxin (including metabolites) in humans. A toxicokinetic model for T2 and HT2 toxin will support future risk assessment based on human biomonitoring studies.

To assess the relation between the T2 and HT2 intake and urinary excretion, sensitive analytical methods are required as the expected T2 and HT2 intake and the total amount excreted is lower than that of DON. If sufficient human data can be obtained, a human urinary excretion factor may be derived that can be used in future HBM studies to estimate the dietary exposure to these mycotoxins in humans.

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6 Annex A

EMBASE search string dd Nov 2020 for nivalenol and fusarenone-X – numbers at the end of te row indicate identified articles

#28. #6 AND #24 AND [humans]/lim	29
#27. #5 AND #24 AND [humans]/lim	30
#26. #6 AND #24	129
#25. #5 AND #24	112
#24. #10 OR #13 OR #18 OR #23	6,762,976
#23. #19 OR #20 OR #21 OR #22	1,382,717
#22. 'biotransformation'/exp OR **'biotransform***':ti	762,658
#21. 'extraction'/exp OR **'extract***':ti	370,361
#20. 'detoxification'/exp OR **'detoxyf***':ti	238,897
#19. 'eliminat*':ti	35,330
#18. #14 OR #15 OR #16 OR #17	6,078,277
#17. 'excretion'/exp OR **'excret***':ti	117,035
#16. 'metabolism'/exp OR **'metabolism'**:ti	5,783,045
#15. 'distribut*':ti	194,867
#14. 'absorption'/exp OR **'absorb***':ti	92,948
#13. #11 OR #12	1,489
#12. 'pbpk':ti OR **'pbbk'**:ti OR (**'pbk'** NEAR/2 'model'):ti)	769
#11. 'adme':ti	723
#10. #7 OR #8 OR #9	782,443
#9. 'toxicokinetics'/exp OR **'toxicokinetic***':ti	12,326
#8. 'biokinetics'/exp OR **'biokinetic model'**/exp OR **'biokinetic***':ti	860
#7. 'pharmacokinetics'/exp OR **'pharmacokinetic***':ti	772,215
#6. #2 OR #4	309
#5. (#1 OR #3) AND [2016-2020]/py	220
#4. 'fusarenon*x':ti	48
#3. 'nivalenol':ti OR **'nivalenol-3-gluco***':ti	212
#2. 'fusarenon x'/exp	301
#1. 'nivalenol'/exp	773

EMBASE search string dd Aug 2021 for T2 and HT2 toxin – numbers at the end of the row indicate identified articles

#14 #12 AND #13	256
#13 #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10	6,852,428
#12 #1 AND #11	562
#11 [2016-2020]/py	8,056,297
#10 'biotransformation'/exp OR **'biotransform*'**:ti	796,857
#9 'pbpk':ti OR **'pbbk'**:ti OR (((**'pbk'** OR **'pbtk'**) NEAR/2 **' model'**):ti)	876
#8 'pharmacokinetics'/exp OR **'toxicokinetics'**/exp OR **'biokinetics'**/exp OR **'pharmacokinetic*'**:ti OR **' toxicokinetic*'**:ti OR **'biokinetic*'**:ti	825,724
#7 'adme':ti OR **'adme'**:ab	4,753
#6 'absorption parameters' OR **'absorb*'**:ti	14,601
#5 'distribution parameters' OR **'distribut*'**:ti	203,767

#4 'elimination' OR **'elimination half-life'** OR **'excretion'**/exp	300,487
#3 'toxicokinetics'/exp	12,301
#2 'metabolism'/exp OR **'metabolism'**:ti	6,098,100
#1 't 2 toxin'/exp OR **'t 2 toxin'** OR **'ht 2 toxin'**/exp OR **'ht 2 toxin'**	2,517

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